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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Aluminium-adjuvants support immunogenic delivery of combined protein-and DNA. based vaccines

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Aluminium-adjuvants support immunogenic delivery of combined protein- and DNA-based vaccines

Field of the invention

The present invention relates to a novel, combined DNA/Protein vaccine formulation comprising nucleic acid molecules and a protein antigen adjuvanted in an mineral-based adjuvant so as to enhance the immunogenicity of both vaccines.

Background of the invention

DNA vaccination is a potent, novel technique to efficiently induce humoral and cellular immune responses. When compared with conventional vaccines, however, DNA vaccines often induce lower antibody titers. Furthermore, rather large amounts of plasmid DNA have to be injected into a mammal to obtain a detectable immune response, e.g. intramuscular injection of 50-100 µg/mouse antigen-encoding plasmid DNA into mice is required to elicit immune responses against the antigen encoded by the DNA vaccine. No, or only barely detectable immune responses are induced when mammals [redacted] with <10 µg plasmid DNA/mouse.

In recent literature various adjuvants have been proposed to enhance the immunogenicity of DNA vaccines.

For example, the interrelated references WO 00/02591, WO 98/35562, and S. Wang et al. *Vaccine* 18:1227-1235 (2000) disclose vaccine formulations comprising nucleic acid molecules and a aluminium phosphate-based or calcium phosphate-based adjuvant provided in a biologically effective concentration so as to improve induction of an immune response subsequent to vaccination. The formulations with calcium- or aluminum phosphate adjuvants prior to intramuscular injection have been shown to facilitate delivery of DNA vaccines, since antibody titers are increased by 10- to 100-fold and the immunogenic dose of DNA is decreased by 10-fold. Although the mechanism by which aluminium phosphate or aluminium hydroxyphosphate exerts this adjuvant effect is not clear, the adjuvant appears to increase the number and affinity of antigen-specific T cells.

WO 99/51269 discloses a DNA vaccine containing a naked DNA incorporating and expressing *in vivo* a nucleotide sequence encoding an antigenic polypeptide and at least an adjuvant compound selected from the class of (meth)acrylic polymers and copolymers of maleic anhydride and alkenyl derivatives, preferably a carbomer or an EMA®.

WO 02/03961 discloses a nucleic acid delivery system comprising nucleic acid molecules encapsulated in biodegradable microspheres, suitable for delivering DNA vaccines, which comprises an adjuvant for modulating the immunostimulatory efficacy said adjuvant comprising an aminoalkyl glucosaminide-4-phosphate (AGP).

There is still a need for further investigation in order to improve the immunogenicity of DNA vaccines. It is noted in this connection that as far as the inventors are aware the possibility of combining DNA nucleotide-based vaccines with conventional protein or adsorbed protein-based vaccines for establishing an efficient combination of these two technologies has hitherto not been explored.

Therefore, an object of the present invention is to provide a combined DNA/protein vaccine with improved immunogenicity as compared to either of the individual vaccines

Summary of the invention

After extensive research and experimentation it has now surprisingly been found that aluminium phosphate- or aluminium hydroxyphosphate-mediated enhancement of the immunogenicity of DNA vaccines was improved by pre-incubating aluminium phosphate or aluminium hydroxyphosphate with at least one suitable protein.

Therefore, the present invention relates to a pharmaceutical formulation comprising (a) a mineral-based, negatively charged adjuvant, in conjunction with at least one protein, and (b) a polynucleotide vaccine encoding at least one antigen, such that introduction of said formulation into a vertebrate host results in expression of a biologically effective amount of said antigen or antigens so as to induce a prophylactic or therapeutic immune response.

In a preferred embodiment of the invention said mineral-based, negatively charged adjuvant is an adjuvant selected from the group of aluminium phosphate-based, aluminium hydroxyphosphate-based, calcium phosphate-based, and calcium hydroxyphosphate adjuvants.

In another preferred embodiment of the invention said at least one protein is selected from either the group consisting of model protein antigens, such as bovine serum albumin, human serum albumin, and lysozyme, or *vaccine protein antigens* intended to induce specific immunity towards infections themselves, (protein antigens such as surface and core protein of HBV) exemplified by, but not limited to core protein of HBV.

In yet another preferred embodiment of the invention said mineral-based, negatively charged adjuvant is pre-incubated by said at least one protein prior to being formulated with said polynucleotide vaccine.

These and other embodiments of the present invention will be outlined in more detail in the following detailed description.

Detailed description of the invention

The term "model protein antigens" as used herein is to define antigens as not intended to induce specific immunity to infections by themselves.

The term "vaccine protein antigens" as used herein refers to antigens intended to induce specific immunity towards infections themselves.

The present invention relates to an improved vaccine formulation comprising nucleic acid molecules and a mineral-based, negatively charged adjuvant provided in a biologically effective concentration so as to promote the effective induction of an immune response directed toward one or more specific antigens encoded by the nucleic acid molecule, the improvement being characterized by a different appearance of the adjuvant in that said adjuvant is pre-incubation, e.g. pre-incubated or pre-mixed, with a suitable protein. The original vaccine formulations on which the improvement is based have been extensively described, *inter alia* in WO 00/2591, the disclosure of which is incorporated herein by reference. Apart from the improvement defined above, any aspect of said disclosure including definitions, preparation of the pharmaceutical formulations, selection and amounts of ingredients, methods of inducing an immune response in an vertebrate host using the pharmaceutical formulations, way of delivery of the formulations to the vertebrate host, the diseases or disorders to be treated by the formulations, etc., apply equally to the present invention.

The present invention is based on the surprising finding that the negatively charged mineral-based phosphates, as exemplified by aluminium phosphate- and aluminium hydroxyphosphate-mediated enhancement of the immunogenicity of DNA vaccines, in particular plasmid DNA vaccines could be further improved by pre-incubating aluminium phosphate or aluminium hydroxyphosphate, or the corresponding calcium salts, with a suitable protein, as exemplified by either a model protein, such as bovine serum albumine (BSA) having low adsorption affinity, and hen egg lysozyme (HEL) having high adsorption affinity, or a vaccine relevant protein antigen, as exemplified by - but not limited to - HBV protein. Accordingly, a protein antigen can be suitably and advantageously co-delivered with DNA vaccines formulated in aluminium phosphate, aluminium

hydroxyphosphate, calcium phosphate, calcium hydroxyphosphate, or another suitable negatively charged mineral adjuvant.

Experimental vaccines were formulated in which low doses (1-5 µg/mouse) of antigen-encoding plasmid DNA together with low doses of either a model protein antigen (BSA), or a vaccine-relevant protein antigen (HBcAg) was mixed with aluminium phosphate or alumium hydroxyphosphate. The immune responses elicited by this polyvalent vaccine against the protein antigen adsorbed to aluminium phosphate were enhanced in the presence of the DNA vaccine, and did not interfere with immune responses against the antigen encoded by the DNA vaccine.

Therefore, protein antigen-containing mineral-based, negatively charged adjuvants, such as aluminium phosphate an aluminium hydroxyphosphate and the corresponding calcium salts can efficiently enhance not only plasmid DNA vaccines but also provides a novel strategy for immunogenic, multivalent combined protein/DNA vaccine delivery.

The invention is further illustrated by the following examples which, however, are not intended to limit the invention in any respect.

Experimental

Immunization of BALB/c mice with protein- or DNA-based vaccines was chosen as the preclinical model because most experience with novel vaccination approaches has been generated in this species.

Standard immunization procedures were used based on the intramuscular injection of either 100 µg plasmid DNA vaccines, or 0.5-5 µg protein antigen.

1. Mixing a protein (antigen) together with a plasmid DNA vaccine to aluminium phosphate or alumium hydroxyphosphate enhances the immunogenicity of the latter

BALB/c mice were immunized i.m. with 5, 50 or 100 µg pCI/S plasmid DNA (encoding the small HBsAg).

The DNA was delivered:

- without vehicle (as 'naked' plasmid DNA)
- with aluminium phosphate
- without protein antigen added
- with (A) BSA or (B) HEL added

The antibody response was determined 4 weeks after a single immunizat

(A) BSA system

group	pCI/S DNA vaccine (µg/mouse)	protein (BSA)	aluminium phosphate	anti-HBsAg serum antibody titer mIU/ml
1	5	-	-	<10
2	50	-	-	978
3	100	-	-	1898
4	5	-	+	458
5	50	-	+	2889
6	5	+	-	<10
7	50	+	-	2756
8	5	+	+	1789
9	50	+	+	4572
10	-	+	-	<10

(B) HEL system

group	pCI/S DNA vaccine (µg/mouse)	protein (HEL)	aluminium phosphate	anti-HBsAg serum antibody titer mIU/ml
1	5	-	-	<10
2	50	-	-	613
3	100	-	-	965
4	5	-	+	549
5	50	-	+	2790
6	5	+	-	<10
7	50	+	-	1473
8	5	+	+	753
9	50	+	+	3317
10	-	+	-	<10

These results allow the following conclusions:

- A dose-dependent antibody responses is induced by a single I.m. injection of the pCI/S DNA vaccine.
- The immunogenicity of the pCI/S DNA vaccine is enhanced when it is mixed before I.m. delivery with aluminium phosphate or alumium hydroxyphosphate.
- When protein antigens (BSA) are mixed with aluminium phosphate or alumium hydroxyphosphate the immunogenicity of the pCI/S DNA vaccine also mixed to aluminium phosphate or alumium hydroxyphosphate is strikingly enhanced.

2. Mixing a protein antigen and a plasmid DNA vaccine with aluminium phosphate

BALB/c mice were immunized I.m. with 5, 50 or 100 µg pCI/LC149 plasmid DNA (encoding a secreted, truncated variant of the HBV core antigen HBcAg).

The DNA vaccine was delivered:

- without vehicle (as 'naked' plasmid DNA)
- with aluminium phosphate
- without protein antigen added with HBsAg added

HBsAg (2 µg/mouse) was delivered.

- without vehicle (as 'naked' protein particles)
- with aluminium phosphate
- without protein antigen added with DNA vaccine added

The antibody response was determined 4 weeks after a single immunization. The results shown in the following table.

group	pCI/LC$_{149}$ DNA vaccine (µg/mouse)	Protein antigen: HBsAg	aluminium phosphate	reciprocal anti-HBc/eAg endpoint antibody titer	anti-HBsAg serum antibody titer mIU/ml
1	5	-	-	<10	<10
2	50	-	-	10.000	<10
3	100	-	-	26.000	<10
4	5	-	+	2.500	<10
5	50	-	+	75.000	<10
6	5	+	-	<10	2187
7	50	+	-	10.000	3210
8	5	+	+	12.500	2630
9	50	+	+	120.000	3540
10	-	+	+	<10	2100

These results allow the folowing conclusions:

- A dose-dependent antibody responses is induced by a single i.m. injection of the pCI/LC$_{149}$ DNA vaccine; the immunogenicity of the DNA vaccine is enhanced when it is mixed before i.m. delivery with aluminium phosphate or alumium hydroxyphosphate
- Protein antigens (HBsAg) and DNA vaccines are mixed with aluminium phosphate or aluminium hydroxyphosphate prime polyvalent immune responses

3. Adsorption of protein antigen (HBsAg) to aluminium phosphate/alumium hydroxyphosphate or aluminium hydoxide

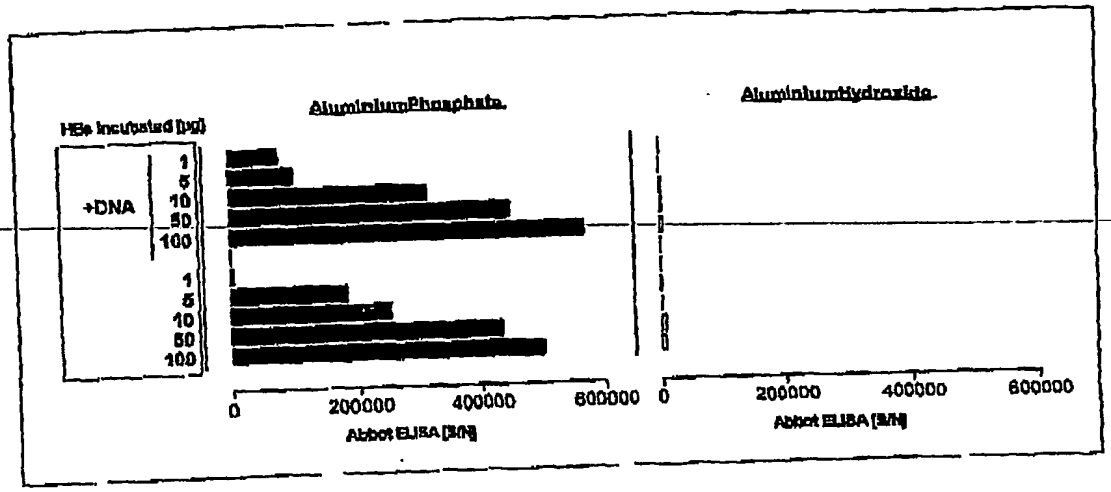
In vitro experiments:

i. 91 µl = 450 µg Al of either aluminium phosphate or aluminium hydroxide were incubated for 24 h at 4°C (under constant shaking) with 100, 50, 10, 5 or 1 µg HBsAg in a total volume of 200 µl; a parallel set of samples was incubated additionally with 50 µg plasmid DNA

ii. the alum was spin down and supernatants were harvested

iii. HBsAg in supernatants (S/N) was measured by the Abbott ELISA

The results are shown in the folowing graph.



HBsAg was readily detected in S/N of mixtures of antigen with aluminium phosphate (even when only low amounts of 1 µg HBsAg were mixed with the alum) while no HBsAg was detectable after incubation with aluminium hydroxide. There was no difference in HBsAg release when plasmid DNA was also mixed (together with HBsAg [illegible]) to the aluminium adjuvant preparation.

These results allow the following conclusions.

- Aluminium phosphate and alumium hydroxyphosphate do not adsorb HBsAg but aluminium hydroxide adsorbs HBsAg efficiently.
- Plasmid DNA has no influence on the adsorption or non-adsorption of protein antigen to aluminium adjuvants.

Claims

1. A pharmaceutical formulation comprising:

(a) a mineral-based, negatively charged adjuvant, in conjunction with at least one protein, and

(b) a polynucleotide vaccine encoding at least one antigen, such that introduction of said formulation into a vertebrate host results in expression of a biologically effective amount of said antigen or antigens so as to induce a prophylactic or therapeutic immune response.

2. A pharmaceutical formulation according to claim 1 wherein said mineral-based, negatively charged adjuvant is an aluminium phosphate-based or an aluminium hydroxyphosphate-based adjuvant.

3. A pharmaceutical formulation according to claim 1 wherein said mineral-based, negatively charged adjuvant is a calcium phosphate-based or a calcium hydroxy-phosphate-based adjuvant.

4. A pharmaceutical formulation according to any one of claims 1 to 3, wherein said at least one protein is selected the group of model protein antigens.

5. A pharmaceutical formulation according to claim 4, wherein said at least one protein is bovine serum albumin, human serum albumin or lysozyme.

6. A pharmaceutical formulation according to any one of claims 1 to 3, wherein said at least one protein is selected from the group of vaccine protein antigens.

7. A pharmaceutical formulation according to claim 6, wherein said at least one protein is surface protein or core protein of HBV.

8. A pharmaceutical formulation according to any one of claims 1 to 7, wherein said mineral-based, negatively charged adjuvant is pre-incubated or subsequently mixed with said at least one protein prior to being formulated with said polynucleotide vaccine.

Abstract of the invention

The present invention relates to a novel, combined DNA and protein antigen vaccine formulation comprising nucleic acid molecules and a mineral-based, negatively charged adjuvant, so as to enhance the immungenicity of the DNA vaccine efficiently enhancing not only plasmid DNA vaccines but also providing a novel strategy for immunogenic, multivalent combined protein/DNA vaccine delivery.